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Differential Association Between MAOA, ADHD and Neuropsychological Functioning in Boys and Girls

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Attention-deficit/hyperactivity disorder (ADHD) is more common in boys than in girls. It has been hypothesized that this sex difference might be related to genes on the X-chromosome, like *Monoamine Oxidase A* (MAOA). Almost all studies on the role of MAOA in ADHD have focused predominantly on boys, making it unknown whether MAOA also has an effect on ADHD in girls, and few studies have investigated the relationship between MAOA and neuropsychological functioning, yet this may provide insight into the pathways leading from genotype to phenotype. The current study set out to examine the relationship between MAOA, ADHD, and neuropsychological functioning in both boys (265 boys with ADHD and 89 male non-affected siblings) and girls (85 girls with ADHD and 106 female non-affected siblings). A haplotype was used based on three single nucleotide polymorphisms (SNPs) (rs12843268, rs3027400, and rs1137070). Two haplotypes (GGC and ATT) captured 97% of the genetic variance in the investigated MAOA SNPs. The ATT haplotype was more common in non-affected siblings ($P=0.025$), conferring a protective effect for ADHD in both boys and girls. The target and direction of the MAOA effect on neuropsychological functioning was different in boys and girls: The ATT haplotype was associated with poorer motor control in boys ($P=0.002$), but with better visuo-spatial working memory in girls ($P=0.01$). These findings suggest that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls and underline the importance of taking into account sex effects when studying ADHD.

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KEY WORDS: attention-deficit/hyperactivity disorder; monoamine oxidase type A (MAOA); sex; neuropsychology; endophenotype

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a strongly genetically determined disorder, characterized by symptoms of inattention, hyperactivity and impulsivity [American Psychiatric Association [APA], 1994]. The disorder is more common in boys than in girls with estimated sex ratios varying between 3:1 and 9:1 [Arnold, 1996; Gaub and Carlson, 1997]. Given these sex differences, it has been hypothesized that genes on the X-chromosome may be important for the pathogenesis of ADHD [Jiang et al., 2001; Lung et al., 2006; Manor et al., 2002]. In contrast to girls, boys do not have a potentially compensatory spare X-chromosome, making them more vulnerable to X-linked diseases. Even though one X-chromosome is inactivated in girls [Ohno et al., 1959], this inactivation is not complete, since a number of genes escape inactivation [Pinsonneault et al., 2006]. Genetic variants in certain X-linked genes may, therefore, have a different impact on cognition and behavior in boys and girls.

An X-linked gene that may show such an effect and may explain sex ratio differences in ADHD is the gene coding for Monoamine Oxidase A (MAOA). This gene is located on the X-chromosome between p11.23 and p11.4 [Das et al., 2006] and escapes X-inactivation in girls [Pinsonneault et al., 2006]. It has 15 exons and codes for a mitochondrial enzyme involved in the pre-synaptic degradation of the monoamines serotonin, norepinephrine and dopamine [Craig, 2007]. The gene is a candidate for ADHD, because it influences the monoaminergic systems that are also etiologically related to ADHD [Das et al., 2006] and MAOA activity can be inhibited by methylphenidate, which also reduces ADHD symptoms [Solanto, 1998]. Several studies have indeed found various polymorphisms in MAOA (like a 30 bp repeat in the promotor region, a GA repeat in intron 2, and a G/T in exon 8) to be associated with ADHD, with odds ratio's around 1.31 and 1.94 [Brookes et al., 2006; Das et al., 2006; Domschke et al., 2005; Guan et al., 2008; Manor et al., 2002]. However, since these studies have focused predominantly on boys, the effects of the gene on ADHD in

Jaap Oosterlaan and Barbara Franke contributed equally to this work.

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girls is still unknown. Only one study separately analyzed results for a small sample of girls with ADHD ($N = 19$) and a larger sample of boys with ADHD ($N = 110$) and reported that the gene was associated with ADHD in both sexes [Manor et al., 2002]. However, this finding is in need of replication.

Another scarcely investigated issue is the relationship between MAOA and neuropsychological functioning. Neuropsychological functions may serve as ADHD-endophenotypes, or intermediate phenotypes of ADHD. These are heritable, continuously distributed traits that are associated with heightened risk for developing a disorder and act as intermediary between genotype and phenotype [Gottesman and Gould, 2003]. Endophenotypes are proposed to be more heritable than phenotypes because they are etiologically "closer" to the disease genes than clinical phenotypes and offer the advantage of a quantitative trait instead of dichotomous entities like DSM diagnostic categories [Gottesman and Gould, 2003]. Focusing on neuropsychological functions in relation to MAOA in ADHD may provide insight into the pathways leading from the gene to ADHD. Given that MAOA influences several monoamines, the neuropsychological effects of this gene may also be diverse. Thus far, only the relationship between MAOA and higher cognitive functions has been reported: MAOA genotype and activity have been found to be related to inhibition [Af Klinteberg et al., 1990–1991; Manor et al., 2002; Meyer-Lindenberg et al., 2006] and memory related cognition [Savitz et al., 2007]. In the current study, MAOA genotype was investigated in relation to both cognitive and motor functions, given that ADHD is frequently associated with deficits in these functions [Halperin and Schulz, 2006]. The cognitive and motor measures studied here have been previously tested and consistently associated with ADHD as endophenotypes [Rommelse et al., 2007a,b,c, 2008a,b].

Thus, the current study set out to examine the relationship between MAOA genotype, ADHD and neuropsychological functioning in both boys and girls. To allow for a robust analysis of the MAOA genotypic effect, a haplotype (combination of alleles transmitted together; Sklar, 2005) based on three single nucleotide polymorphisms (SNPs) was used in the analysis. This haplotype had shown nominal association with ADHD in the main International Multicenter ADHD Genetics (IMAGE) project sample [Brookes et al., 2006] of which the current study targets a subsample.

MATERIALS AND METHODS

Subjects

Participants were recruited in the Dutch part of the IMAGE study that aims to identify genes that increase the risk for ADHD using QTL linkage and association strategies [Kuntsi et al., 2006]. Families with at least one child with the combined subtype of ADHD (proband) and at least one additional sibling (regardless of possible ADHD-status) participated. For the current study, the sample was split by sex, resulting in the participation of 265 boys with ADHD, 89 male non-affected siblings, 85 girls with ADHD, and 106 female non-affected siblings. All children were between the ages of 5 and 19 years and were of European Caucasian descent. Participants were excluded if they had an IQ < 70, a diagnosis of autism, epilepsy, brain disorders or known genetic disorders, such as Down syndrome or Fragile-X-syndrome, which can mimic some of the ADHD symptoms.

The screening procedures and measures for phenotyping have been described previously [Brookes et al., 2006]. Briefly, the diagnosis of ADHD was based on screening questionnaires (parent and teacher Conners' long version rating scales and parent and teacher Strengths and Difficulties Questionnaires [SDQ]) [Conners, 1996; Goodman, 1997] and a semi-structured interview [Parental Account of Children's Symptoms [PACS], Taylor, 1986]. Scores were considered clinical if T -scores were ≥ 63 on the Conners subscales (DSM-IV Inattention, Hyperactive-Impulsive, and ADHD Total) and >90 th percentile on the SDQ subscale Hyperactivity. For diagnostic purposes, data of the questionnaires and the PACS were subjected to a standardized algorithm to derive each of the DSM-IV ADHD symptoms, providing operational definitions for each behavioral symptom [Brookes et al., 2006].

Neuropsychological Tasks

The ten neuropsychological tasks used in this study have been described and analyzed elsewhere [Rommelse et al., 2007a,b,c, 2008a,b] and are presented in Table I. Based on previous results [Rommelse et al., 2007a,b,c, 2008a,b], the variable for each task, which showed the most optimal results in the endophenotypic analyses, was chosen for analysis. All variables were normalized and standardized using a Van der Waerden transformation (Statistical Package for the Social Sciences [SPSS] version 14).

TABLE I. Description of the Neuropsychological Tasks

Task	Aim of measurement	Dependent variable
Executive/cognitive tasks		
Stop task ^a	Inhibition	Stop signal reaction time (SSRT)
Shifting attentional set ^b	Inhibition and cognitive flexibility	Percentage of errors
Time test ^a	Time reproduction	Accuracy (total absolute deviation between stimulus and response)
Visuo-spatial sequencing ^c	Visuo-spatial working memory	Number of correct targets in the correct order
Digit span ^c	Verbal working memory	Digit span backwards
Motor tasks		
Pursuit ^c	Motor control under continuous adaptation	Precision
Tracking ^c	Motor control without continuous adaptation	Precision
Tapping ^d	Self-generated motor output	Variability in tapping rate
Baseline speed ^d	Motor output as response to external cue	Variability in reaction times
Motor timing ^d	Timing of motor output	Variability in reaction times

^aRommelse et al. [2007a].

^bRommelse et al. [2007b].

^cRommelse et al. [2007c].

^dRommelse et al. [2008a].

^eRommelse et al. [2008b].

DNA Extraction, MAOA Genotyping, and Haplotype Estimation

An elaborate description of methods for DNA extraction is provided elsewhere [Brookes et al., 2006]. Briefly, DNA was extracted directly from blood samples or cell lines at Rutgers Cell line and DNA repository in the US. Three SNPs in *MAOA* (rs12843268 [intron 5, G/A], rs3027400 [intron 9, G/T] and rs1801291 [exon 14, now known as rs1137070, C/T]) were selected as these had shown nominal association with ADHD in a larger sample of IMAGE (respectively $P = 0.049$, $P = 0.049$, and $P = 0.020$), in which the entire *MAOA* gene-region had been investigated using tagSNPs [Brookes et al., 2006]. The SNPs were genotyped using the Illumina Golden Gate AssayTM (Illumina Inc., San Diego, CA). Additional families, which had been included in IMAGE at a later stage and had not been described in the paper by Brookes et al. [2006], were genotyped for the three SNPs using ABI SNPlex [Tobler et al., 2005] as part of a replication study (unpublished data). In total 178 (74.8%) of the ADHD families in the current study underwent genotyping, the numbers of samples genotyped for each SNP are shown in Table III.

Linkage disequilibrium (LD) values of the *MAOA* SNPs were determined using HAPLOVIEW [Barrett et al., 2005] and ranged between 0.873 and 0.99. Haplotypes were estimated using the haplo.em function implemented in the haplo.stats package [Sinnwell and Schaid, 2005], which computes maximum likelihood estimates of haplotype probabilities. Posterior probabilities of haplotype pairs for each subject were also computed to account for the fact that there may be more than one pair of haplotypes that are consistent with the observed marker genotypes. Haplotype association analyses were done using the haplo.score function [Schaid et al., 2002]. Briefly, this package computes score statistics to test associations between haplotypes and a wide variety of traits, including binary, and allows adjustment for other determinants. This analysis was corrected for multiple testing by applying the $simulate = TRUE$ parameter in haplo.score which gives simulated P values. These simulated haplotype score statistics are calculated from a permuted re-ordering of the trait (ADHD status) and covariates (in this case *MAOA* SNPs). We used 1,000 permutations for all the analyses. Finally, missing SNP genotypes were inferred using the observed genotype data from the rest of the sample using the haplo.em function. In this way, the number of missing genotypes was reduced to zero.

Since our sample is composed of family data, we initially estimated the overall haplotype frequencies using the parental data only. Thereafter, we separately estimated the children haplotype frequency in the groups of affected and non-affected children. Six different haplotypes were present in the parental and children samples: GGC, ATT, AGC, ATC, AGT, and GGT (Table II). Haplotypes GGC and ATT captured 97.14% of the genetic variance in the investigated *MAOA* SNPs. Therefore,

TABLE II. *MAOA* Haplotype Distribution within the Sample

Haplotype	Parents (N = 477)	Affected children (N = 350)	Non-affected children (N = 195)
GGC	0.63806	0.67455	0.60781
ATT	0.3278	0.28676	0.3819
AGC	0.01995	0.02325	0
ATC	0.00664	0.00759	0.00684
GGT	0.00446	0	0.00345
AGT	0.00308	0.00786	0

further analyses report only on these two haplotypes. Analyses were carried out for the dataset including the imputed genotype data as well as for the dataset without these data.

Data Analysis

Since *MAOA* is X-linked, we used the genotypes of mothers to test for Hardy-Weinberg equilibrium (HWE) using the Markov-Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3. No deviations from HWE were detected for the three SNPs ($df = 2$, P -values between 0.479 and 0.982).

Haplotype frequency was compared between the group of affected and non-affected participants in order to find differences in frequency distribution. The association of *MAOA* with the neuropsychological measures was analyzed using a linear mixed model with *MAOA* as factor (two haplotype groups for boys: GGC and ATT; three diplotype groups for girls: GGC_GGC, GGC_ATT, and ATT_ATT) and family structure as random effect. In addition, a possibly moderating effect of age was taken into account by adding the effect of age group (two groups split by median age: children <11.5 years and adolescents >11.5 years) into the model as well as the interaction between *MAOA* and age. The rationale for this approach was based on previous findings in this sample, showing that associations between the dopamine transporter gene (*DAT1*) [Rommelse et al., in press] and the dopamine receptor 4 gene (*DRD4*) [Altink et al., submitted] with neuropsychological measures were different in children and adolescents. Correction for multiple comparisons according to the False Discovery Rate (FDR) controlling procedure was applied to the analyses with a q -value setting of 0.05 [Benjamini and Hochberg, 1995]. Following Cohen's guidelines [Cohen, 1988], effect sizes were defined in terms of the percentage of explained variance: 1%, 9%, and 25% were used as cut-off to define small, medium, and large effects. These figures translate into η^2 -values of 0.01, 0.06 and 0.14.

RESULTS

Characteristics of the sample are described in Table III. No age differences were present between the groups, but affected

TABLE III. Sample Characteristics

	Girls with ADHD	Female non-affected siblings	Boys with ADHD	Male non-affected siblings
N	85	106	265	89
Age in years (SD)	12.2 (3.1)	11.5 (3.8)	11.9 (2.7)	11.3 (3.5)
NADHD subtype (%)				
Inattentive	13 (15.3)	—	15 (5.7)	—
Hyperactive-impulsive	12 (14.1)	—	6 (2.3)	—
Combined	60 (70.6)	—	244 (92.1)	—
N genotyped (%)				
SNP1 (rs12843268)	63 (74.1)	85 (80.2)	196 (74.0)	60 (67.4)
SNP2 (rs3027400)	57 (67.0)	80 (75.5)	184 (69.4)	54 (60.7)
SNP3 (rs113707) ^a	71 (83.5)	90 (84.9)	222 (83.8)	67 (75.3)

^aPreviously known as rs1801291.

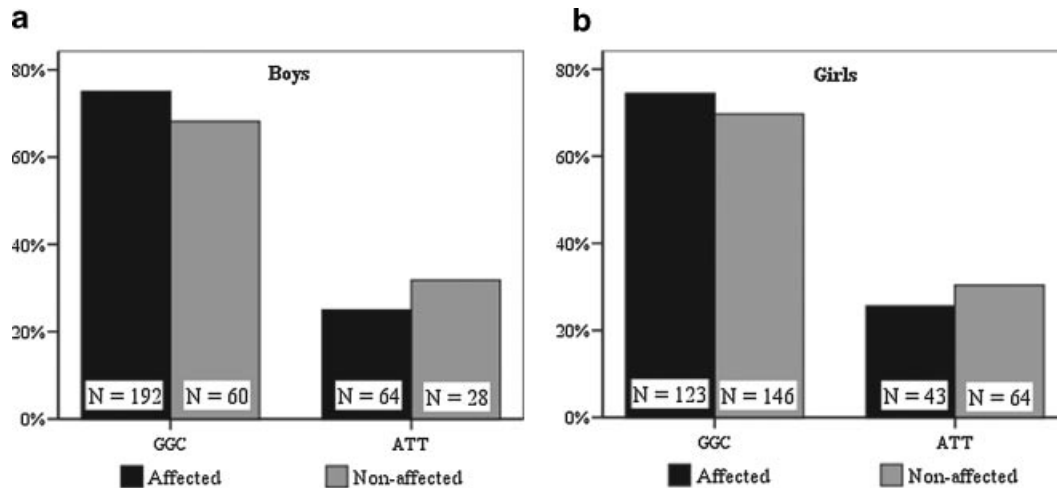


Fig. 1. Frequencies of the two most common *MAOA* haplotypes (GGC and ATT) in affected and non-affected boys (a) and girls (b).

boys had more often the combined subtype compared to affected girls (92.1% vs. 70.6%), whereas the inattentive and hyperactive-impulsive subtypes were more common in girls than boys (15.3% vs. 5.7% and 14.1% vs. 2.3%, respectively).

The ATT haplotype was more common in non-affected siblings (38.2%) compared to affected participants (28.7%) ($P = 0.025$). The frequency of the GGC haplotype was higher in affected participants (67.5%) compared to non-affected siblings (60.8%), though this difference was not significant ($P = 0.095$). These effects were apparent in both boys and girls (Fig. 1).

In boys, *MAOA* haplotype had a significant effect on the Pursuit task, measuring motor control under continuous adaptation ($F(1, 311.5) = 9.62, P = 0.002, \eta_p^2 = 0.03$), and a nominally significant effect on the Tracking task, measuring motor control without continuous adaptation ($F(1, 300.8) = 4.66, P = 0.032, \eta_p^2 = 0.02$). Boys having the GGC haplotype performed better than boys with the ATT haplotype (Fig. 2). These effects were comparable in children and adolescents, since the interaction between *MAOA* and age were not significant for Pursuit or Tracking ($F(1, 336.0) = 0.39, P = 0.53$ and $F(1, 333.0) = 0.51, P = 0.48$, respectively).

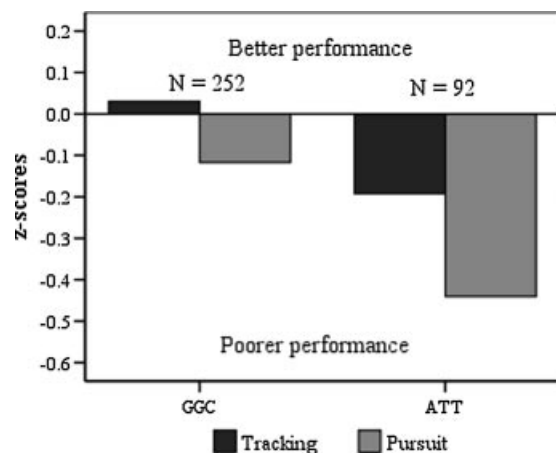


Fig. 2. Relationship between *MAOA* haplotypes and Pursuit (motor control under continuous adaptation) and Tracking (motor control without continuous adaptation) in boys.

No main effects of *MAOA* haplotype or interaction effects between *MAOA* and age on other neuropsychological measures were found in boys (data available on request).

In girls, *MAOA* diplotype had a nominal significant effect on the Visuo-Spatial Sequencing task, measuring visuo-spatial working memory ($F(2, 184.0) = 4.77, P = 0.01, \eta_p^2 = 0.05$). A nominal significant linear effect was present ($P = 0.01$) with girls having the ATT ATT diplotype performing best, girls with the GGC GGC diplotype performing poorest, and girls with the GGC ATT diplotype intermediately (Fig. 3). This effect was comparable for children and adolescents, since the interaction between *MAOA* diplotype and age was not significant ($F(2, 184.0) = 1.85, P = 0.16$). No additional main effects of *MAOA* haplotype or interaction effects between *MAOA* and age on other neuropsychological measures were found in the girls. Findings were similar, when analyses were repeated including only the children for whom haplotype data were available and did not need to be estimated (data available on request). In addition, findings were similar for affected and non-affected children, since post-hoc analysis of the interaction between *MAOA* haplotype and diagnosis was not significant for any of the measures in boys or girls (data available on request).

DISCUSSION

We set out to examine the relationship between *MAOA* genotype, ADHD and neuropsychological functioning in both boys and girls. Based on three SNPs, six different haplotypes were observed in our sample, of which two were common (GGC in 65.18% and ATT in 31.96%). All other, rare haplotypes (frequencies of 2.3% and below) were excluded from analysis. Both in boys and girls, the ATT haplotype was more common in non-affected siblings compared to affected participants, suggesting that this haplotype may have a protective effect against developing ADHD. The GGC haplotype had a somewhat higher frequency in affected versus non-affected participants, though not significantly. This latter finding is in line with the findings in the IMAGE study sample: The alleles of the SNPs within the haplotype individually as well as part of a haplotype showed

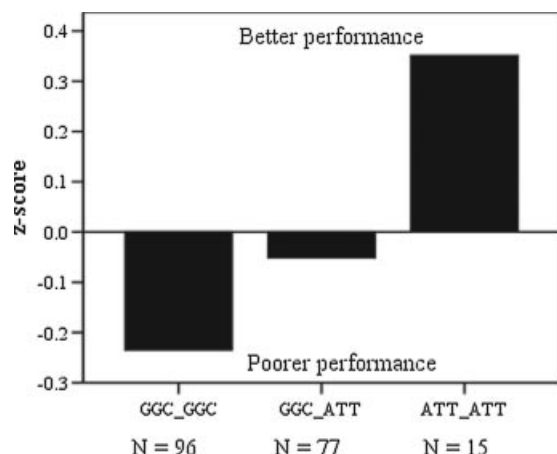


Fig. 3. Relationship between *MAOA* diplotype and visuo-spatial working memory in girls.

overtransmission to ADHD-affected children [Brookes et al., 2006]. These findings suggest the relationship between *MAOA* and ADHD to be present in both boys and girls and are in line with previous findings in a substantially smaller sample of girls [Manor et al., 2002]. However, these findings do not shed light on the large sex differences in the risk of developing ADHD.

In contrast to the absence of a moderating effect of sex on the relationship between *MAOA* and ADHD, a moderating effect of sex was present on the relationship between *MAOA* and neuropsychological functioning. In boys, the ATT haplotype was associated with motor control. In girls, the ATT haplotype was associated with visuo-spatial working memory. *MAOA* mainly influences the metabolism of serotonin [Craig, 2007]. Serotonin, in turn, has an influence on a diverse range of brain functions, amongst others on motor functions: Motor regions of the brain are innervated by serotonin projections and the involvement of serotonin systems in the control of movements has clearly been shown in animal studies [Oades, 2007]. However, serotonin has also been shown to play a role in cognitive functions, like learning and (working) memory through its localization in "cognitive pathways" (such as the hippocampus and frontal cortex) [Cifariello et al., 2007; Oades, 2007]. Thus, the finding that *MAOA* genotype influences cognitive as well as motor functioning in ADHD is not surprising, but the observation that sex moderates these effects is. This may be related to biological differences between males and females in serotonin neurotransmission, such as differences in serotonin receptor binding potentials [Jovanovic et al., 2008], differences in number of serotonin receptor types, differences in brain and blood levels of serotonin, and differences in the speed of serotonin synthesis [Cosgrove et al., 2007]. These serotonergic sex differences are believed to underlie sex differences in the prevalence and clinical presentation of serotonin-associated psychiatric conditions, such as depression and anxiety [Cosgrove et al., 2007]. It is, therefore, feasible that the effect of *MAOA* through serotonin levels on neuropsychological functions may not necessarily be comparable between boys and girls with ADHD, as is suggested by our findings. Based on these results, it is possible that serotonin related medication used to treat ADHD symptoms, such as tricyclic antidepressants and monoamine oxidase inhibitors, may have a differential effect on ADHD in boys and girls. However, the current results are too preliminary to draw such conclusions.

In keeping with these data on sex differences in serotonin neurotransmission, a moderating effect of sex was found on the

relationship between *MAOA* and neuropsychological functioning. Not only the target (motor control versus visuo-spatial working memory), but also the direction of the effect was different in boys and girls. In both sexes, the ATT haplotype appeared to have a protective effect against ADHD in this study sample. However, in boys, the ATT haplotype was conversely associated with poorer neuropsychological performance, whereas in girls the ATT haplotype was associated with better performance. This finding may be related to differential effects of serotonin levels on behavior and cognition [Oades, 2007]. Decreased serotonin levels in the cerebrospinal fluid have been associated with poorer aggression control (which may be viewed as decreased behavioral inhibition), yet also with better ability to inhibit on experimental paradigms (which may be viewed as increased cognitive inhibition) [Oades, 2007]. Thus, similar serotonin levels may produce opposite behavioral and cognitive effects [Oades, 2007]. If one would translate this to the current findings, motor control may be viewed as belonging more closely to the behavior domain and visuo-spatial working memory more closely to the cognitive domain.

Differential sex effects of *MAOA* on brain functions have also previously been reported. For example, only in males, but not females, was an association found between a low-expressing variant of *MAOA* and dysregulated amygdala activation and increased functional coupling with ventromedial prefrontal cortex [Buckholtz et al., 2008]. Furthermore, differential sex effects of *MAOA* on clinical manifestation have been described for a number of psychiatric disorders, such as for obsessive compulsive disorder [Karayiorgou et al., 1999], panic disorder [Deckert et al., 1999], mood disorders [Lin et al., 2000], and pathological gambling [Ibañez et al., 2000]. The effect of *MAOA* on brain and behavioral functions may thus be moderated by sex in a wide spectrum of functioning. Our findings of both the target and direction of the *MAOA* effect on neuropsychological functioning differing between the sexes, suggests that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls and underlines the importance of taking into account sex effects when studying ADHD.

A final point of discussion is the finding that we found an effect of *MAOA* on visuo-spatial working memory (in girls), but not on other executive functions, such as inhibition and verbal working memory. This might be hypothesized as being related to subtle differences in the underlying neurotransmitter systems mediating the executive functions. Serotonin, amongst other neurotransmitters, relates to visuo-spatial working memory [Luciana et al., 2006], but not, or to a lesser extent, to inhibition [Chamberlain et al., 2006] or verbal working memory [Kugaya et al., 2003]. Given that *MAOA* mainly influences the metabolism of serotonin [Craig, 2007], this may explain the selective effect of *MAOA* on visuo-spatial working memory and not inhibition or verbal working memory. However, the current findings need replication before firm conclusions can be drawn on the specificity of *MAOA* in relation to visuo-spatial working memory.

Our findings should be viewed in the light of several limitations. First of all, group sizes were relatively small, as well as were the haplotype frequency differences between the groups of affected and non-affected siblings. Therefore, our findings need to be replicated before firm conclusions can be drawn. Second, the group of affected boys and girls differed in the distribution of ADHD subtypes: The boys had more often the combined subtype compared to the girls, which may have influenced the differential effect of sex on the findings. However, this is unlikely, since repeating the analyses including only boys and girls with the combined subtype, we observed similar, and in girls even more significant, results. Third, the effect of *MAOA* on neuropsychological functioning

appears not to be profound. In only three of ten neuropsychological measures was a small effect found, of which only one survived correction for multiple testing. Importantly, since the female sample was smaller than the male one, the small effect sizes have limited the power to detect effects of genotype in females. Fourth, the neuropsychological measures used here are by no means representative of the full domain of neuropsychological functions and tasks relevant for ADHD. The current findings need replication before firm conclusions may be drawn on the differential effects of MAOA on neuropsychological functioning in boys and girls.

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